Original Research Article

OCCURRENCE OF MULTIDRUG RESISTANCE *ESCHERICHIA COLI* AND OTHER BACTERIA SPECIES ASSOCIATED WITH URINARY TRACT INFECTION IN TWO GEOPOLITICAL ZONES OF ONDO STATE, NIGERIA

Abstract

Urinary tract infection (UTI) is a common bacterial infection known to affect different parts of the urinary tract of both male and female. *Escherichia coli* has been found to be responsible for causing 80% to 90% of the infection. An investigation was carried out to determine the prevalence of bacteria, especially *E.coli* implicated in UTI, and to ascertain their antibiotics susceptibility pattern. Early morning mid-stream urine samples were collected from 250 patients aged 18 to 60 years, between March and July of 2016 from 5 major Hospitals in the study location. The isolates were identified using standard microbiological methods and susceptibility tests were carried out using ten antibiotics. Results showed that 65(30.7%) of the isolates were *E. coli*. *Pseudomonas aeruginosa* 45(21.2%), *Klebsiella pneumoniae* 42(19.8%), *Staphylococcus aureus* 32(15.1%) and *Proteus mirabilis* 28(13.2%). The percentages of resistance of *E. coli* isolates to antimicrobial agents were chloramphenicol (64.9%), sparfloxacin (59.5%), ciprofloxacin (73.0%), septrin (73.0%), amoxacillin (91.9%), augmentin (83.8%), gentamycin (48.7%), perfloxacin (40.5%), ofloxacin (40.5%) and streptomycin (54.1%). The need for constant antimicrobial susceptibility surveillance by health managements system that will help clinicians to provide safe and effective therapy is advocated.

Key words: Uropathogen, *Escherichia coli,* Urinary tract infection

INTRODUCTION

Urinary tract infection is one of the most frequent bacterial infectious diseases that affect both inpatient and outpatients around the world [8], and is one of the most common diseases that affect people of all ages from neonate to adulthood and globally [12, 30]. UTI is more common among females than male with about 60% of women having at least one episode in their lifetime [21]. This is due to shorter urethra in females which is more readily transverse by microorganisms [53]. The highest incidence of UTI occurs mostly in child bearing age due to the fact of high increase in sexual activity and aging. Gupta [23] reported that about 150 million people are diagnosed with urinary tract infection each year.

Escherichia coli have been found to be responsible for 90% of the first urinary tract infections in young women [21]. They are known as a component of the intestinal microflora of human or warm blooded animals [9], which can exist as commensal, it also exist in a symbiotic state

37 providing resistance against pathogenic organisms or it can be pathogenic and cause diseases of
38 the intestinal and extra intestinal sites

39 Over 150 million people experienced an episode of UTI yearly and this is costing the world
40 economy over 6 billion US dollars in treatment and workloss [23]. Studies that have being
41 carried out in Enugu, Yola, Zaria and Ile-Ife showed that the same etiological agent was isolated
42 in Nigeria. The prevalence rate of urinary tract infection in Yola was 67.2% and 77% in Enugu
43 [20]. Olowu [41] reported an incidence of 28.1% in a population of 2780 outpatients at Lagos
44 University Teaching Hospital while Aiyegoro [2] reported 11.96% in Ile-Ife. The common
45 bacteria associated with urinary tract infections aside *E. coli* are *Klebsiella*, *Pseudomonas*,
46 *Proteus* and also the Gram positive organism like *Stappylococci* sp, *Enterococci* sp. *Candida*
47 *albicans* has been isolated in patients with underlying physiological debilitation [35].

48 Commensal strains of *E. coli* can also cause diseases in immunocompromised hosts, the
49 pathogenic and commensal *E. coli* are classified into four main phylogenetic groups: A, B1, B2
50 and D [13]. The phylogenetic group A and B1 consists mainly of the commensal strains that are
51 found in large intestine of humans and animals, but they do not naturally carry any known
52 virulence factor [28]. Whereas, the phylogenetic group B2 and to a lesser extent the group D
53 consist of pathogenic strains that normally carry virulence–associated genes, which are mostly
54 associated with extra-intestinal diseases. The pathogenic *E. coli* strains causes enteric or
55 diarrhoegenic disease, urinary tract infection and blood infection. The aim of this study is to
56 isolate and identify microorganisms that are associated with urinary tract infection and to assess
57 the antibiotic susceptibility pattern of the isolates.

58 **MATERIALS AND METHODS**

59 **Sample collection**

60 A total of 250 urine samples were collected from volunteers (between the ages of 18 and 60
61 years) that visited the General Hospital, Ikare-Akoko; Federal Medical Center Owo; Adekunle
62 Ajasin University Health Center Akungba-Akoko and Union Diagnostic Center, Akure, Ondo
63 State, Nigeria between Jan and May in 2016.

64 **Culture and identification of isolates**

65 Each sample was inoculated on MacConkey agar, Eosin Methelene Blue (EMB) agar and
66 Cystine Lactose Electrolyte Deficient (CLED) agar plates and incubated at 37^{0}C for 24 hours.
67 The identification and characterization of isolated bacteria were done by Gram staining, followed
68 by microscopic examination and biochemical tests such as indole production, citrate utilization
69 and phenol red lactose broth tests.

70 **Susceptibility test**

Antimicrobial sensitivity testing of all the isolates was performed using disk diffusion method according to [14]. Clinical samples were inoculated on Mueller Hinton plates and spread evenly to form a lawn culture, after which, the antibiotic discs were placed on the surface and incubated at 37^0C for 24 hours. The antibiotics used include: cotrimoxazole (30ug), chloramphenicol (30ug), sparfloxacin (10ug), ciprofloxacin (10ug), amoxacillin (10ug), augmentin (30ug), gentamycin (10ug), perfloxacin (10ug), ofloxacin (10ug) and streptomycin (30ug).

RESULTS

A total of 212 (84.8%) of urine samples yielded significant growth, out of 155 (62%) females that participated, 150(96.8%) showed positive culture, while 62(65.3%) showed positive culture out of 95(24.8%) males that participated (Table 1). Five different uropathogens were isolated *E.coli* was predominant 65(26%) followed by, *Pseudomonas aeruginosa* 45(18.0%), *Klebsiella pneumonia* 42(16.8%), *Staphylococcus aureus* 32(12.8%), and *Proteus mirabilis* 28(11.2%). Thirty eight (15.2%) samples had no bacteria growth (Table 2). The frequency of bacteria isolated from male and female volunteers was shown in Table 3.

Distribution according to age group revealed that the highest rate of infection occurred in age group 18-45 and the least infection was recorded in volunteers above 46 years (Table 1). The antimicrobial drugs used on the uropathogenic *E.coli* isolated showed that ofloxacin (40.5%) and perfloxacin (40.5%) were the most efficient drugs of choice *in vitro*. This was because they showed high efficacy on the isolates than any other antimicrobials used in this study, this was followed by gentamycin (48.7%). The bacteria showed high degree of resistance to amoxicillin (91.9%), augmentin (83.8%), ciprofloxacin (73.0%), cotrimoxazole (73.0%), chloramphenicol (64.9%) sparfloxacin (59.5%) and streptomycin (54.1%) (Table 4) *Pseudomonas aeruginosa* isolated in this study were resistant to chloramphenicol, sparfloxacin, cotrimoxazole, amoxicillin, augmentin, perfloxacin and streptomycin, but were sensitive to gentamycin, ofloxacin and ciprofloxacin (Table 4). The resistance pattern of *K. pneumonia*, *S. aureus* and *P. mirabilis* were also shown in Table 4, the zone of inhibition was 15 to 40mm.

Table 1: Age and Sex distribution of volunteers with significant bacteriuria

Age (years)	Males	Females	Total	Total Percentage
18-45	39	95	134	63.2%
46-60	23	55	78	36.8%
Total	62	150	212	100%
	29.2%	70.8%	84.8%	

N= 250

Table 2: Prevalence of microorganisms isolated from urine of patients

Organisms isolated	Number of isolates	Percentage (%)
Escherichia coli	65	26.0
Pseudomonas aeruginosa	45	18.0
Klebsiella pneumonia	42	16.8
Staphylococcus aureus	32	12.8
Proteus mirabilis	28	11.2
No bacteria growth	38	15.2
Total	**250**	**100**

Table 3: Frequency of isolated bacteria from positive cultures of volunteers

Bacteria isolates	Female	Male	Total (%)
Escherichia coli	49(75.4%)	16(24.6%)	65(30.7)
Pseudomonas aeruginosa	32(71.1%)	13(28.9%)	45(21.2)
Klebsiella pneumonia	28(66.7%)	14(33.3%)	42(19.8)
Staphylococcus aureus	26(81.3%)	6(18.8%)	32(15.1)
Proteus mirabilis	12(42.9)%	16(57.1%)	28(13.2)
Total	**147(69.3%)**	**65(30.7%)**	**212(100)**

Table 4: Antimicrobial sensitivity pattern showing resistance to bacterial agents

Isolates	CH	SP	CPX	SXT	AM	AU	CN	PEF	OFX	S
Escherichia coli	64.9	59.5	73.0	73.0	91.9	83.8	48.7	40.5	40.5	54.1
Pseudomonas auriginosa	65	80	30	55	70	65	25	50	30	65
Klebsiella pneumonia	50	57.1	50	78.6	78.6	71.4	64.3	35.7	14.3	85.7
Staphylococcus aureus	58.3	66.7	50	58.3	33.3	41.7	41.7	50.0	25.0	66.7
Proteus mirabilis	80	50	30	70	80	80	20	60	30	70

CH=Chloramphenicol, SP=Sparfloxacin, CPX=Ciprofloxacin, SXT=Septrin , AM=Amoxacillin, AU=Augmentin, CN=Gentamycin , PEF= Perfloxacin , OFX=Ofloxacin, S=Stremptomycin

DISCUSSION

Out of 250 patients (155 females and 95 males between the ages of 18 – 60years), that participated in this study, 212(84.8%) urine samples had significant bacteriuria. This was higher than the study on profile of urinary tract infection and quinolones resistance among *E.coli* and *Klebsiella* spp isolated by Namratha [37], who reported the prevalence of 62%. Also prevalence of 67.2% was reported in Yola [20], 60% reported in Lafia [30], 22% in Ibadan [40], 77.9% in Enugu [33] and 35.5% in Jos [19] all in Nigeria. The high prevalence observed might be due to factors like peer group influence, promiscuity, pregnancy, low socio-economic status that are common among Nigerian young men and women [30].

This study revealed that *E. coli* was one of the predominant organisms incriminated in urinary tract infection. Out of 212 positive isolates *E. coli* had 65(30.7%) followed by *P. aeruginosa* 45(21.2%), *K. pneumonia* 42(19.8%), *S. aureus* 32(15.1%) and *P. mirabilis* 28(13.2%). The pattern and frequency of occurrence of the bacterial isolates found in this study is similar to those reported by other workers. Alausa and Onile [5] reported in their study that *Escherichia coli* was the most commonly isolated pathogens in significant bacteriuria. Also, Bishara [10] in Israel agreed with this statement, the authors reported that *E. coli* was responsible for 52% of cases of

urinary tract infection, *Klebsiella* spp.(14%), *Proteus* species (95%) and *Enterococcus* spp. was 4 %. A variant result was observed in a study in Ile-Ife Southwestern Nigeria by Okonko [41] where *S. aureus* 11(28.9%) was second to *E. coli* 13(42.1%), *Klebsiella* spp 7(18.4%) and *Pseudomonas* spp 2(5.3%). A variant was also observed in a tertiary hospital in Abuja, Central Nigeria, where Iregbu and Nwajiobi-Princewill [25] proved that *E.coli* and *Klebsiella* spp were the two predominant uropathogens 323(37%) and 202(25%) respectively, *P. aeruginosa* 75(8.4%), *Proteus* spp 67(7.5%) and *S. aureus* 60(6.8%). The same trend of *E. coli* leading other uropathogens was reported by [36, 56].

The frequency of urinary tract infections in female was 150(70.8%) and the occurrence in male was 62(29.3%). This finding was in agreement with studies carried out by [24, 41]. This also correlates with other studies by [55, 29] where urinary tract infection was higher in females compared to males. Anochie [7] reported a predominance of female patients in a study carried out to determine the influence of instruction about the method of urine collection and storage on the prevalence of urinary tract infection. Similar findings were reported by [42]. The author reported that urinary tract infection was more frequent in women than men [20, 1, 45]. Okafor [39] reported 20.7% cases of urinary tract infection in males which was similar to 29.3% cases reported in this study.

The higher incidence of urinary tract infection in females has been attributed to the shorter female urethra and its close proximity to the anus that make it easier for enteric flora to colonize this area [15, 49]. Other contributory factors may include the use of contraceptives, childbirth and menopause [44, 17].
The highest rate of infection (UTI) was recorded in volunteers between the age group 18-45 and the least infection (UTI) was recorded in volunteers above 46 years, this is in agreement with the study conducted by [6]. The high incidence of urinary tract infection between the ages of 18-45 years is due to high sexual activities and multiple sex partners among the age group.

The antibiotic resistant *E coli* isolated from urinary tract infections are on high increase and this is a serious problem. Many of the isolated strains are multi-resistance [4, 32]. In this study 54.1% of the *E coli* isolates were noted to be multiple resistant, they are resistant to more than six antibiotics. The antibiotic sensitivity test of this study shows that ofloxacin and perfloxacin were the most efficient antibiotics in vitro testing followed by gentamycin and sparfloxacin, meanwhile most of the organisms are highly resistance to amoxicillin followed by augmentin ciprofloxacin, septrin and chloramphenicol. Sensitivity test in this study revealed that ofloxacin and perfloxacin have the highest efficacy in vitro (59.5%) and (59.5%) respectively, this is in agreement with [26] where ofloxacin had (70.5%) as the most effective among antimicrobials. In this study 91.9% resistance to amoxicillin was found, and this is in agreement with the report of [34] The authors reported 95% resistance to amoxicillin, also Datta [16] reported 88.57% and 100% resistance to amoxicillin was reported by [55].
The percentages of the pathogens resistance varied between 91.9% and 40.5%. In this study, the isolates have demonstrated extremely high percentage of multidrug resistance phenotype. The

178 rate of resistance to Septrin found is higher than those reported by others [54, 57]. Septrin or
179 trimethoprim alone has been used widely in treating urinary tract infections caused by *E. coli* for
180 decades this study shows low efficacy of septrin also Ciprofloxacin resistance in this study is
181 found to be higher than that reported in previous studies [48].

182 Current study shows that resistance to Ciprofloxacin is on high increase and if urgent measures
183 are not taken to put the situation under control the need for search of new drugs to combat
184 bacterial infection will arise in Nigeria, meanwhile few ciprofloxacin resistance strains were
185 found in other countries [17].

186 The high level of resistance seen in Amoxicillin, Augmentin, Septrin, Ciprofloxacin,
187 Chloramphenicol and Sparfloxacin is most likely due to selective pressure that results from
188 uncontrolled and inappropriate use of these agents in our hospitals and in this country as a whole,
189 this is promoted by poverty, lack of awareness about the usage of drugs, lack of antibiotic policy
190 and also the readily availability of antibiotics sold over the counter in Nigeria. The high rate of
191 antimicrobial resistance has pose a problem in the medical sector, 54.1% of our *Escherichia coli*
192 population were noted to be multiple resistance.

193 In this study *Pseudomonas aeruginosa* isolated were resistance to chloramphenicol, sparfloxaxin,
194 septrin, amoxicillin, augmentin, perfloxacin and streptomycin, but were sensitive to gentamycin,
195 ofloxacin and ciprofloxacin. Olowu and Oyetunji [43] also reported multi-resistance in
196 *Pseudomonas aeruginosa* in their study of nosocomial urinary tract infection. *Klebsiella*
197 *pneumonia* showed high level of resistance to most of the antibiotics used a resistance of 78.6%
198 was seen against amoxicillin, 85.7% were seen against streptomycin, 71.4% were resistance to
199 augmentin and 64.3% to gentamycin while 14.3?% were resistance to ofloxacin this result was
200 similar to that reported by [2].

201 *Staphylococcus aureus* had a resistance pattern of 58.3% to Chloramphenicol, 66.7% to
202 sparfloxacin, 50% to ciprofloxacin, 58.3% to cotrimoxazole,50.0% to perfloxacin, 66.7% to
203 streptomycin this is contrary to [3] report which presented a report in which 67.3% of
204 Staphylococcus spp were resistance to cloxacillin, 64.9% to amoxicillin,51.8% to augmentin,
205 70.2% to tetracycline,48.8% to erythromycin, 36.9% to cotrimoxazole, 11.9% to
206 chloramphenicol and 1.8% to gentamycin in a study in Ibadan Nigeria

207 **CONCLUSION**

208 The choice of drugs in the treatment of urinary tract infection (UTI) is quite narrow due to the
209 wide -scale resistance that the UTI pathogens exhibited. *E. coli* remains the most frequent
210 bacteria isolate. There is the need for constant antimicrobial sensitivity surveillance which will
211 help clinicians to provide safe and effective therapy. The role of personal hygiene to curtail its
212 spread cannot be overemphasized.

213 **COMPETING INTERESTS**
214 Authors have declared that no competing interests exist.

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